DEVICE AND METHOD FOR HANDLING SMALL VOLUME SAMPLES AND/OR REACTION MIXTURES

The present invention concerns a device and a method for the simultaneous handling of a multitude of small volume samples, and in particular novel means and a method for compressing or condensing a set of samples such as a set of samples on a microtitre plate from one format to a less space-demanding one.

Background of the invention

Biochemical reactions are routinely carried out in so-called microtitre plates – sheets of moulded plastic that typically contain eight rows and twelve columns of tiny wells, each of which capable of holding a few millilitres or less of the reaction mixture. This is known as the 8 by 12 or 96-well format. The 96-well format is widely used, not only in biochemical operations, such as analysis, diagnostics, small scale synthesis and the like, but also in fields like high throughput screening and combinatorial chemistry.

Lately, a tendency towards the development of more compact microtitre plate formats has been seen. A plate with 16 rows and 24 columns is already in use and generally known as the 384-well format. Further development can be expected, leading to the introduction of a 32 by 48 plate, or a 1526-well format. While the handling of microtitre plates becomes more and more automated, there often remains one or several steps where a component, for example the sample to be analysed, is added manually. An initial step of transferring samples from a serial format, e.g. patient samples in separate test tubes, to a parallel format, e.g. to separate wells in a microtitre plate, is by necessity involved in practically all applications. Naturally, manual handling is incompatible with a too far reaching miniaturisation. In order to be practically manageable, the receiving microtitre plate cannot have too high resolution. A very dense format will become physically unmanageable from the user's point of view and the risk of mix-ups and contamination increases.

In addition to the above, the 96-well format is widely used and accepted by the laboratories, both in research and other applications. Auxiliary equipment has been designed to accommodate this format. Importantly, the historical and wide-spread use of the 96-well format means that vast amounts of old patient samples, compounds for drug development etc are stored in the 96-well format. In order to subject these to new analyses and investigations, for example in the field of drug development using high throughput screening, the format has to be compressed. Presently, such reformation, compression or condensation is done by pipetting robots. They are however complicated and costly apparatuses, and they often exhibit a less than satisfactory yield.

In view of the above, there remains a problem of how to efficiently, simply and practically adapt the 96-well format to the present and future developments involving highly dense microtitre plates, such as the 384- and 1536-well format.

Summary of the invention

The above problem is solved by the device and method as defined in the attached claims. The inventive device allows easy conversion between different microtitre plate formats, thus adding flexibility and making possible the sequential use of apparatuses designed for different formats. Other applications and benefits of the invention will be described in or become evident from the description and examples below.

Short description of the drawings

The invention will be described in closer detail below, with reference to the accompanying drawings in which

Fig. 1 shows schematically the compression steps from the 96-well format to the 384- and further to the 1536-well format;

Fig. 2 shows an adapter according to claim X in perspective;

Fig. 3 shows the above adapter in cross section along the line III;

Fig. 4 shows the above adapter in cross section along line IV; and

Fig. 5 shows schematically how four adapters, two of each (denoted A and B, respectively) are assembled together.

Description

The following description will refer to "microtitre plates" in general, which is hereby defined as comprising any device capable of receiving a multitude of samples in separate and/or discrete locations or so called wells. The term "grid" is used to define the spatial distribution of said wells, e.g. their distribution in rows and columns, on the microtitre plate. Further, the term "upper" is used to define the surface of the adapter having a grid corresponding to the less dense format. Consequently, the term "lower" is used to define the surface of the adapter having a grid corresponding to the more dense format. These definitions do however not eliminate the possibility that the adapter according to the invention is used in any other orientation.

The term "small volume reactions" includes any chemical reaction conducted in volumes below 100 µl. Typical biochemical reactions, intended to fall within this definition,

are immunological determinations, histological determinations, enzymatic determinations, biochemical diagnosis of diseases, determinations of paternity, various determinations in forensic medicine, various operations in analysis and synthesis applications. Typical chemical reactions, intended to fall within this definition are reactions in the screening and synthesis of pharmaceuticals, for example high throughput screening, combinatorial chemistry, analysis, determination of environmental pollutants etc.

Below, the terms "donating" and "receiving" are used to distinguish between the less dense microtitre plate, from which a sample, reagents etc. is "donated" or transferred to a denser, "receiving" microtitre plate.

The adapter according to the invention is a three-dimensional body having openings on one surface, said openings spaced according to the less dense donating format, and a corresponding amounts of openings on its reverse surface, said openings being connected by individual channels. Importantly, the relative position of each opening on one surface is identical to the position of the corresponding opening on the reverse surface. In other words, the grid of the wells on the upper surface corresponds to the grid of microtitre plates of this less dense format, which is subject of compression. The grid of the openings on the lower surface in its turn corresponds to the grid of the more dense, receiving format.

An adapter for compressing the 96-well format into the 384-well format typically has 24 wells, arranged in 6 rows and 4 columns, on its upper surface. Correspondingly, there are 24 openings on its lower surface, arranged in 6 rows and 4 columns. Importantly, the grid on the upper surface is identical to the grid of a 96-well microtitre plate, and the grid on the lower surface is identical to the grid of a 384-well microtitre plate.

In addition to the grid being identical, it is important that the adapter is designed to fit tightly to the openings of the donating microtitre plate. The individual wells on the adapter will be designed to fit to the wells of the donating microtitre plate, sufficiently well to prevent leakage or cross-contamination when the combined adapter and donating plate is turned up-side-down. This can be achieved by providing the adapter with protruding rims, around each well, protruding cylinders that fit into the donating wells, gaskets, flanges etc.

Naturally the adapter can consist of a full 8 by 12 upper surface in the 96-well format and a corresponding 8 by 12 lower surface in the 384-well format. It is however preferred that the adapter is manufactured in two parts being a mirror image of each other. When put into use, two of each part are assembled as shown in Fig. 3.

In order to compress a conventional 8 by 12 microtitre plate (the 96-well format) into a more dense format, e.g. a 16 by 24 plate (the 384-well format) four adapters are assembled as illustrated in Fig. 3. The set of four adapters can be arranged on top of a receiving microtitre plate, e.g. one corner or a quarter of a microtitre plate in the 384-well format. The samples and reagents, as desired, are then dispensed in the wells of the adapters, either manually or using equipment developed for the 96-well format. The adapters, together with the receiving microtitre plate or quarter thereof, are then placed in a centrifuge and subjected to a centrifugal force sufficient to transfer all the contents of the upper wells, through the channels, into the receiving denser format. The adapters can then be removed and discarded and the samples, being in the 384-well format, processed further or analysed using equipment adapted to this format. Possible further steps for the contents transferred to the denser format include analysis, storage or emptying or a combination thereof. Analysis steps may include the addition of a further reagent and the analysis itself can encompass photometric determinations, such as measuring the fluorescence intensity of the mixture.

When using a quarter of a 384-well microtitre plate, i.e. a microtitre plate having 8 rows and 12 columns, four such quarters have to be put together in order to constitute a complete 384-well microtitre plate with 16 rows and 24 columns. When a complete 384-well microtitre plate is used as the receiving format, the procedure described above has to be repeated four times.

For compressing the 384-well format into the 1536-well format, adapters are manufactured according to the above principle. A 384-well plate is first compressed into a subset of a 1536-well plate, whereupon these subsets are assembled into a full 1536-well plate.

According to one embodiment of the invention, the channels connecting the upper and lower openings on an adapter are made so narrow, that the sample and/or reaction mixture cannot pass the channels unless the adapter is subjected to a certain centrifugal force. Similarly, the channels can be closed by an inert substance which is either solid or viscous at normal analysis temperature, but which melts or turns less viscous at an increased temperature within a range, tolerable for the sample and reagents. In this way the user can have control over the reaction, for example by adding a sample plus reagent in the less dense format and, at a desired moment, end the reaction by centrifuging the mixture into th receiving format, possibly containing an agent quenching the reaction. Using a solid or viscous "plug" the user can decide when to transfer the contents to the denser format by regulating the temperature during centrifugation.

According to another embodiment of the invention, the channels connecting the upper and lower openings on the adapter are provided with constrictions or filter means for separating the sample and reagent mixture from auxiliary components, such as particulate matter or reagent capillaries. Particulate matter, e.g. silica particles, glass beads, latex beads etc is frequently used in extraction, purification and separation operations. An adapter provided with constrictions or filter means would therefor function as a convenient means for separating the particulate matter from the reaction mixture, simultaneously as the separate samples are transferred to a denser format. When the adapter itself functions as the reaction vessel, the filter means constitute a convenient way to simultaneously separate the particulate matter from the reaction mixture and emptying the contents into a denser format for further analysis or subsequent reactions.

In the receiving format, the mixture is then subjected to possible further treatment or analysis. Consequently, the adapter itself can function as a microtitre plate, being the receptacle of samples and reagents, with the further benefit of allowing the reaction mixture to be transferred into a denser format at any time desired by the user.

The adapter or device according to the invention is preferable made of a suitable thermoplastic. Examples of suitable materials include, but are not limited to, polypropylene (PP), polystyrene (PS), polyethylene (PE), high density polyethylene (HDPE), polycarbonate (PC), polyacetate (PA), poly-methyleene-methacrylate (PMMA) and poly-vinylidene-fluoride (PVDF). The choice of material is not only governed by thermal and chemical considerations, but also economic considerations such as material costs, production technology etc. One suitable method of production is injection moulding. Vacuum die-casting is another possible method of production. The adapter is of course manufactured under conditions rendering it sterile and free from contaminants, which possibly could influence the reaction or reactions it is intended for.

According to a preferred embodiment of the invention, the device comprises physical characteristics aiding in the correct positioning of the device in relation to surrounding devices and in relation to the receiving microtitre plate. Such physical characteristic can comprise velts, grooves, colour codes and pins, with corresponding members in the adjacent device. In practise this can be realised by coloured fields, forming a pre-determined pattern when the devices are correctly assembled. Another example is that hte device is equipped with pins on at least one side, said pins fitting in corresponding holes on at least one side of the adjacent device, when the devices are correctly assembled. Similarly

velts or raised details can be provided, said velts or raised details fitting in corresponding grooves or depressions on the adjacent device when correctly assembled.

According to another embodiment, the device according to the invention comprises predispensed reagents, preferably in reagent cartridges or capillaries which are emptied by centrifugation. These can be single- or multi-compartment cartridges or single- or multi-lumen capillaries. According to this embodiment, reagents can be added to the samples or reaction mixtures before or during the compression or transferral to a denser format. This is achieved through the fact, that reagent cartridges and capillaries can be made to release their content at predefined temperatures or centrifugal forces. For more information on reagent cartridges, see e.g. WO 98/10866. A method according to the present invention includes both the possibility, that the adapter is delivered with suitable reagent cartridges or capillaries in place and the possibility, that these are added to the wells of the adapter after addition of the sample or reagent mixture that is to be compressed to a denser format. In any case, it is preferred that the channels in the adapter, connecting the openings at the upper surface, corresponding to a less dense format, and the openings at the lower surface, corresponding to a more dens format, are designed not to allow the passage of the reagent cartridge or the reagent capillary.

According to yet another embodiment, the devices according to the invention fits into a frame, said frame also being part of the invention. The frame is designed to fit the receiving microtitre plate and to hold the devices in an orientation and position in relation to each other and in relation to the receiving microtitre plate as to guarantee flawless transfer of the sample or reagents from one format to the other.

The adapter and methods of its use, according to the present invention, offer the user numerous benefits. The device functions as an interface between manual handling of the traditional 96-well format and the automated handling of the newer 384-well format and more dense formats. The device makes it possible to transfer samples and reaction mixtures from one format into a more dense one with little or no risk for error, as the alignment of the corresponding wells is an automatic, "built-in" feature of the adapters. Further, the possibility of conducting an exactly determinable part of a reaction in the adapter, after which the reaction mixture is transferred into the receiving format, offers many advantages.

Presently used pipetting robots first remove the sample by suction, frequently leaving a residual volume in the well, and then dispense it, leaving a small residual volume in the pipette. Compared to this, the inventive device and method is superior, both in regard of the initial costs and sample economy.

The inventive device and method is particularly suited for supplying old samples, stored in serial format or on 96-well plates, to the newly developed methods and apparatuses used in high throughput screening and combinatorial chemistry. The device and method of the present invention constitute a convenient interface between manual and automated operations in all types of small volume reactions, e.g. in fields like diagnostic analysis, biochemical analysis and high throughput screening and combinatorial chemistry.

Examples

Example 1.

Four devices according to the invention are assembled in a frame, and placed over the first quadrant of a 16 by 24 microtitre plate. Samples are manually pipetted into the wells of the devices, now resembling a conventional 8 by 12 microtitre plate. This way the first 96-samples are compressed to the 384-well format. The process is repeated by first assembling four devices over the second, third and fourth quadrant of the receiving 16 by 24 plate.

Example 2.

Four 96-well microtitre plates are filled with samples and reagents in a conventional manner and subjected to temperation in a thermo shaker, built for this format. Four devices according to the invention are assembled in a frame and placed over the first quadrant of a 16 by 24 microtitre plate. The first 96-well plate is emptied in the assembled devices and the reaction mixtures thus transferred and compressed to the 384-well format. The process is repeated with new devices, assembled in lots of four, corresponding to the second, third and fourth quadrant of the receiving 16 by 24 plate.

Example 3.

Four adapters assembled to form a 96-well grid on their combined upper surfaces, are supplied with reagent cartridges, for example single- or multilumen capillaries, containing one or several reagents. (For information on reagent cartridges, see e.g. WO 98/10866) The adapters are connected, up-side-down to a conventional 96-well microtitre plate, forming a tight seal between the openings of the wells on the 96-well microtitre plate and the openings of the adapter. This combination is then turned and connected to the receiving, denser format, e.g. a 384-well microtitre plate and placed in a centrifuge. When reaching a predetermined speed, the reagent cartridge or single- or multilumen capillaries are emptied of their contents and at another, predetermined speed, the entire content of the former 96-well plate and the reagents are transferred into the receiving, 384-well plate.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.
